EXPERIMENTAL STUDIES ON THE EFFECT OF RIBOFLAVIN FOLLOWING THE INTRAVENOUS ADMINISTRATION OF FAT EMULSION

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EXPERIMENTAL STUDIES ON THE EFFECT OF RIBOFLAVIN FOLLOWING THE INTRAVENOUS ADMINISTRATION OF FAT EMULSION

by

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I. INTRODUCTION

The smooth process of metabolism of administered fat is obviously necessary in order to make effective the parenteral nutrition of fat administered intravenously in the form of emulsion.

The concept of the process of the fat metabolism in vivo generally recognized at present is considerably different from that of Knoop's β-oxidation theory. That is, fatty acids become active form by combination of their carboxyl radical with coenzyme A (CoA) and enter into the Fatty Acid Cycle (LYNEN) and then are oxidized to β-hydroxy acids and further to β-keto acids. Then they combine with CoA and become acetyl-CoA. It was explained that this acetyl-CoA is the active form of acetic acid and can be directly condensed with oxalo-acetic acid and then enter into KREBS' Tricarboxylic Acid Cycle (T. C. A. Cycle) and be oxidized to carbon dioxide and water.

Consequently the fatty acid chain is gradually oxidized by loss of two carbon atoms progressively. Many enzyme systems participate in this oxidation process just as with other nutrients. Today, with many enzymes such as Flavoprotein, Pyridine Nucleotide, Aconitase, CoA is regarded as important.

It has become clear that all these enzymes participating in fat metabolism are contained in mitochondria and have respectively pantothenic acid, riboflavin and nicotinic acid (water soluble vitamins) as their principal components and further that aconitase is activated by vitamin C. HASHINO and OSA of our laboratory have

pointed out the necessity of administering these vitamins concomitantly with intravenous administration of fat emulsion. HASHINO studied this problem from the standpoint of the behavior of keton bodies, OSA from the standpoint of economy of protein. While they demonstrated the necessity of vitamins indirectly, the present study has been an attempt to study experimentally directly the behavior of riboflavin in organs and blood in the case of intravenous administration of fat emulsion.

II. EXPERIMENTAL MATERIALS AND PROCEDURE

1. Experimental Materials

The fat emulsion: 15 per cent sesame-oil emulsion which contains 7 per cent glucose is used principally.

Experimental animals: Adult rabbits weighing about 2 kg are used after being fed a standard diet and showing constant body weight and nutrition. As the standard diet bran, 120g, raw radish leaves, 120g and water, 125 cc were used daily. Bran and radish leaves contained respectively 20 γ and 79 γ riboflavin in 100g.

2. Experimental Procedure

The measurement of the total riboflavin in organs was made by YAGI's method, and the measurement of riboflavin as both free riboflavin and ester-form was made by FUJITA's method. In addition the measurement of the riboflavin in blood and urine was made by a modified FUJITA's method.

The outline of measurement is as follows.

Procedure : ……

(i) Extraction. Several methods of extraction are known which make use of methanol, acetone and Taka-diastase etc.

In the present study YAGI's hot-water extraction was used : Extirpated organs were cut into pieces of red bean size and heated 3 to 5 minutes in hot water to 80°C. in order to destroy the action of the enzymes and to degenerate the protein. The material was then ground by homogenizer, a fixed quantity of water added and the final product heated 15 minutes at 80°C.

(ii) Light Resolution. In order to convert riboflavin to lumiflavin, a 200W. electric lamp was used at a distance of 20 cm for 30 minutes.

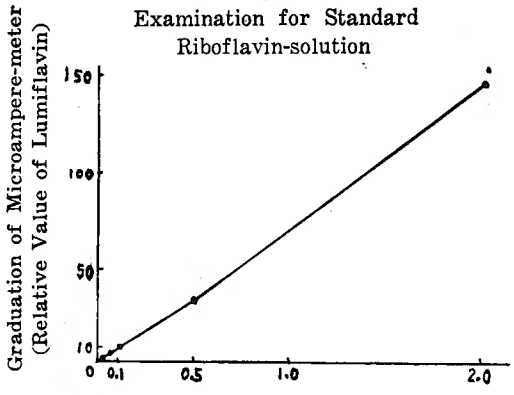
(iii) Chloroform Extraction and Measurement of Fluorescence. After extraction of lumiflavin by chloroform the grade of fluorescence was measured by fluorophotometer (made by PLATZ & BAUER Co.) within the limits of proportional concentration of lumiflavin. (Fig. 1, 2, 3.)

(IV) Removal of Interfering Fluorescent Substances. Some materials other than lumiflavin contain fluorescent substances which are extracted by chloroform in acid solution and interfere with the correct measurement. Therefore, especially in the case of urine, previous extraction with chloroform and oxidation with potassium permanganate were done in order to remove fluorescent substances other than lumiflavin.

(V) Reagents. Chloroform and benzylalkohol are used after distillation if they are fluorescent.

3. Experimental Preparations.

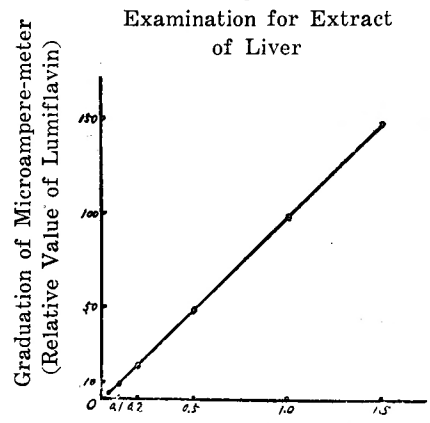
Fig. 1
Examination for Standard Riboflavin-solution



Quantity of Riboflavin (γ)

2γ	151.5	(147.5)
1	77.5	(73.5)
0.5	40.5	(36.5)
0.1	11.0	(7.0)
0.05	7.5	(3.5)
0.01	4.5	(0.5)
Blank (0.01γ)	4.0	

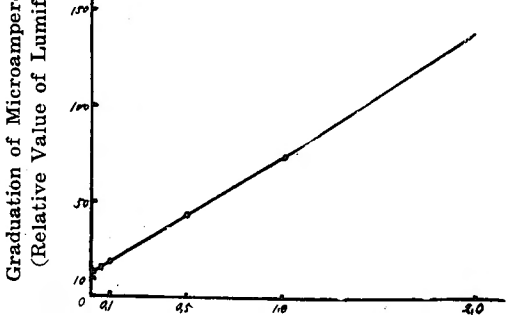
Fig. 2
Examination for Extract of Liver



Extract of Liver (cc)

1.5cc	150	(148)
1.0	100	(98)
0.5	50	(48)
0.2	20	(18)
0.1	10	(8)
0.05	5	(3)
Blank (0.05cc)	2	

Fig. 3
Examination for Extract of Liver Added with Riboflavin



Quantity of Riboflavin Added to Extract of Liver (γ)

2γ	140	(136.5)
1	77	(73.5)
0.5	46.5	(43.0)
0.1	22	(18.5)
0.05	19	(15.5)
0.01	16.5	(13.0)
Blank (0.01γ)	3.5	

In this study riboflavin-5′-phosphate as riboflavin, *l*-ascorbic acid as vitamin C, niacin amide as nicotinic acid, calcium pantothenate as pantothenic acid and thiamin hydrochloride as vitamin B_1, each in aqueous solution were used

III. EXPERIMENTAL RESULTS AND DISCUSSION

1) Concentration of Riboflavin in Organs and Blood of Healthy Adult Rabbits.

Healthy adult rabbits are used after being fed the above mentioned standard diet and after showing constant body weight and nutrition. These rabbits were killed by exsanguination and total riboflavin in their organs and blood was measured. The results are shown in Table 1.

Some individual differences are shown, but the concentration of riboflavin is highest in the liver, next in the kidney, then in heart, lung and spleen in order. When the fat emulsion is administered intravenously, fat globules are first phagocytized by phagocytes of lung (pulmonary alveolar phagocytes), Kupffer's stellate cells of the liver and reticuloendothelial cells of the spleen, the neutral fats being changed to phospholipids in these cells. If triglycerides of short chain fatty acids

Table 1
Concentration of Total Riboflavin of Normal Rabbits

No. of Rabbits	Liver	Kidney	Heart	Lung	Spleen	Blood
	(γ/gm)					(γ/cc)
1.	33.24	22.43	12.35	5.31	3.78	0.143
2.	35.12	23.42	12.86	6.11	4.11	0.154
3.	34.38	22.31	12.21	5.71	3.64	0.147
4.	31.78	22.03	12.01	5.42	3.42	0.135
5.	32.49	21.87	11.95	5.10	3.12	0.131
Average	33.40	22.41	12.28	5.53	3.61	0.142

are used in the fat emulsion, phospholipids produced by intravenous administration enter chiefly into parenchymal cells of the liver. Here their numerous fatty acids are oxidized to ketone bodies, and they are transported by the blood to the kidney, muscles and other tissues except the liver and then finally oxidized. On the other hand, if triglycerides of long chain fatty acids are used in the fat emulsion, phosholipids produced by intravenous administration can enter directly into not only parenchymal cells of the liver but other tissue cells, and then their fatty acids are oxidized to carbon dioxide and water. However, it is thought that long chain fatty acids entering parenchymal cells of the liver can not be totally oxidized to carbon dioxide and water, but a part of them are converted inevitably to keton bodies and are finally oxidized in tissues other than the liver such as kidney and muscles etc. as in the case of short chain fatty acids. The results of studies by ASADA, IZUKURA, SHIROTANI, OSA and HASHINO of our laboratory have confirmed this fact.

Therefore, as evident in Table 1, it is very interesting to note that the more active organ in fat metabolism is the greater the quantity of its riboflavin content. Likewise in the case of carbohydrate metabolism, it is well known that liver and heart contain a great quantity of riboflavin which has the biochemical function of promoting the accumulation of liver and muscle glycogen from administered glucose or lactic acid produced in the body as COLLAZO and BAYO have shown.

Riboflavin is divided into free-form and ester-form, the latter being further divided into Flavin Adenine Dinucleotide (F. A. D) and Flavin Mononucleotide (F. M. N.). According to YAGI's finding, when free riboflavin (F. R.) is adminsitered to animals (rats), whether parenteral or per os, it always changes to F. M. N. in mucous membrane of small intestines and then F. M. N changes to F. A. D. in kidney and liver.

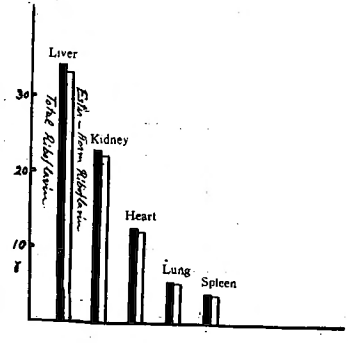
Today it is recognized that riboflavin in the body exists mainly as F. A. D. with smaller amounts of F. M. N. and only a slight amount of F. R. being present. Riboflavin can function in the body only in the form of F. A. D. and of F. M. N. Therefore, the ester-form of riboflavin was measured in organs and blood of adult healthy rabbits. The results are shown in Table 2. Riboflavin in the body of rabbits is mostly in ester-form and very little as F. R., as precursors have shown. Fig. 4 illustrates the proportion of total riboflavin and ester-form in organs and

Table 2
Concentration of Ester-form Riboflavin of Normal Rabbits

No. of Rabbits	Liver	Kidney	Heart	Lung	Spleen	Blood
	(γ/gm)					(γ/cc)
1.	32.54(0.82)	21.86(0.51)	12.25(0.15)	5.14(0.12)	3.68(0.13)	0.157(0.02)
2.	34.42(0.91)	22.74(0.60)	12.75(0.13)	5.97(0.13)	3.84(0.12)	0.143(0.01)
3.	30.47(0.87)	21.62(0.58)	11.87(0.16)	5.11(0.11)	3.13(0.14)	0.132(0.01)
4.	32.58(0.92)	20.67(0.64)	12.41(0.17)	5.42(0.13)	3.76(0.12)	0.134(0.01)
5.	35.14(0.81)	23.15(0.71)	12.31(0.14)	6.12(0.15)	4.12(0.15)	0.158(0.02)
Average	33.03(0.87)	22.01(0.61)	12.32(0.15)	5.55(0.13)	3.71(0.13)	0.145(0.01)

( ) : Concentration of Free Riboflavin (F. R.).

Fig. 4
Riboflavin Proportion in Organs of Rabbit



blood.

2. Concentration of Riboflavin in Organs and Blood of Healthy Adult Rabbits Following a Single Intravenous Administration of Fat Emulsion.

It is known that the consumption of riboflavin increases following administration of fat per os. Then, how does riboflavin behave in the body when fat emulsion is administered intravenously?

Utilizing the fact that an adult Japanese ingests daily an average of 0.5g fat per kg of body weight, 15 per cent sesame-oil emulsion was injected in a dosage of 3.3cc per kg of body weight into the vena auricularis of rabbits. Groups of five rabbits each were killed by exsanguination 6, 12 and 24 hours after intravenous administration, and then the concentration of riboflavin was measured in organs and blood.

Table 3
Concentration of Total Riboflavin in Organs and Blood Following a Single Intravenous Administration of Fat Emulsion.
(Values Obtained 6 Hours after Injection)

No. of Rabbits	Liver	Kidney	Heart	Lung	Spleen	Blood
	(γ/gm)					(γ/cc)
1.	29.64	22.41	12.75	3.14	3.12	0.125←0.133
2.	30.25	23.25	13.00	4.05	3.52	0.111←0.131
3.	28.78	22.65	12.42	4.67	3.15	0.125←0.132
4.	31.05	23.12	12.34	4.88	3.50	0.130←0.135
5.	29.74	21.87	12.57	4.25	3.72	0.133←0.141
Average	29.89	22.66	12.62	4.18	3.40	0.125←0.134
Control	33.16	21.87	12.47	5.72	3.54	0.142 *

* : Before Injection

In view of findings of other investigators of our laboratory that the addition of methionin greatly facilitates metabolism and that reticuloendothelial cells phagocytize fat globules and convert neutral fat to phospholipids, *l*-methionin in a dosage of 0.5mg per kg of body weight was routinely used during intravenous administration of the fat emulsion. Because the fat emulsion contained 7 per cent glucose, 0.23g glucose per kg of body weight, in the same volume of distilled water as the fat emulsion was injected into control rabbits.

(a) Values Obtained 6 Hours after Injection

The concentration of total riboflavin and ester-form in organs and blood of rabbits 6 hours after simultaneous injection of fat emulsion and *l*-methionin is shown in Tables 3 and 4.

Table 4

Concentration of Ester-form Riboflavin in Organs and Blood Following a Single Intravenous Administration of Fat Emulsion.
(Values Obtained 6 Hours after Injection)

No. of Rabbits	Liver	Kidney	Heart	Lung	Spleen	Blood
	(γ/gm)					(γ/cc)
1.	29.41(0.81)	21.34(0.53)	12.61(0.13)	3.86(0.13)	3.51(0.11)	0.125(0.01)←—0.131(0.01)
2.	28.61(0.76)	21.14(0.55)	12.85(0.16)	4.26(0.14)	3.02(0.12)	0.113(0.01)←—0.121(0.01)
3.	30.15(0.78)	22.14(0.62)	12.34(0.15)	4.83(0.13)	3.41(0.11)	0.132(0.02)←—0.138(0.02)
4.	28.62(0.91)	20.75(0.68)	11.88(0.15)	4.76(0.11)	3.14(0.14)	0.128(0.01)←—0.134(0.01)
5.	30.16(0.95)	22.31(0.70)	12.34(0.18)	4.37(0.15)	3.12(0.13)	0.134(0.02)←—0.151(0.02)
Average	29.39(0.84)	21.54(0.62)	12.40(0.15)	4.41(0.13)	3.32(0.12)	0.126(0.01)←—0.135(0.01)
Control	32.58(0.92)	21.86(0.68)	12.48(0.14)	5.72(0.13)	3.52(0.11)	0.138(0.01) *

* : Before Injection
( ) : Concentration of Free Riboflavin (F. R.)

Table 5

Concentration of Total Riboflavin in Organs and Blood Following a Single Intravenous Administration of Fat Emulsion.
(Values Obtained 12 Hours after Injection)

No. of Rabbits	Liver	Kidney	Heart	Lung	Spleen	Blood
	(γ/gm)					(γ/cc)
1.	30.12	22.17	12.25	4.41	3.85	0.142←—0.150
2.	29.58	21.18	11.48	4.73	3.09	0.131←—0.140
3.	31.68	20.48	12.67	4.32	3.65	0.121←—0.133
4.	29.86	22.56	11.58	4.87	3.86	0.112←—0.135
5.	31.47	21.76	12.15	4.76	3.91	0.125←—0.141
Average	30.54	21.63	12.03	4.62	3.67	0.126←—0.139
Control	31.38	22.31	12.27	5.42	3.61	0.131 *

* : Before Injection

(b) Values Obtained 12 Hours after Injection

The concentration of riboflavin 12 hours after injection differs very little from that after 6 hours. The total riboflavin in liver and blood decreased as shown in Table 5, and also that of kindney and heart tended to decrease slightly.

In the case of control rabbits administered only glucose, the concentration of riboflavin in liver and blood decreased slightly.

Table 6

Concentration of Eser-form Riboflavin in Organs and Blood Following a Single Intravenous Administration of Fat Emulsion.

(Values Obtained 12 Hours after Injection)

No. of Rabbits	Liver	Kidney	Heart	Lung	Spleen	Blood
	(γ/gm)					(γ/cc)
1.	29.41(0.73)	21.61(0.52)	12.10(0.15)	4.28(0.13)	3.72(0.11)	0.130(0.02)←0.142(0.02)
2.	28.31(0.69)	20.64(0.55)	11.57(0.18)	4.15(0.15)	3.12(0.12)	0.135(0.02)←0.151(0.02)
3.	30.74(0.81)	21.48(0.64)	11.84(0.16)	4.86(0.11)	3.85(0.15)	0.121(0.01)←0.133(0.01)
4.	28.11(0.84)	20.12(0.71)	12.30(0.14)	4.01(0.13)	2.96(0.14)	0.115(0.01)←0.128(0.01)
5.	30.52(0.91)	21.15(0.63)	12.32(0.15)	4.57(0.15)	3.41(0.11)	0.133(0.02)←0.158(0.02)
Average	29.42(0.79)	21.00(0.61)	12.02(0.16)	4.37(0.13)	3.41(0.13)	0.127(0.02)←0.142(0.02)
Control	32.86(0.85)	22.41(0.61)	12.21(0.15)	5.51(0.13)	3.62(0.12)	0.132(0.01) *

* : Before Injection

( ) : Concentration of Free Riboflavin (F. R.)

(c) Values Obtained 24 Hours after Injection

The concentration of total riboflavin 24 hours after injection is shown in Table 7. The decrease of total riboflavin in liver is especially marked. Riboflavin in

Table 7

Concentration of Total Riboflavin in Organs and Blood Following a Single Intravenous Administration of Fat Emulsion.

(Values Obtained 24 Hours after Injection)

No. of Rabbits	Liver	Kidney	Heart	Lung	Spleen	Blood
	(γ/gm)					(γ/cc)
1.	27.13	24.31	12.48	5.27	3.54	0.131←0.142
2.	24.59	20.12	11.76	4.87	3.39	0.143←0.150
3.	26.41	21.83	12.89	5.10	3.10	0.115←0.134
4.	26.20	20.10	11.34	5.05	3.41	0.125←0.133
5.	28.76	22.31	12.50	5.12	3.21	0.138←0.150
Average	26.62	21.73	12.19	5.08	3.33	0.130←0.142
Control	33.14	22.42	12.27	5.53	3.61	0.142 *

* : Before Injection

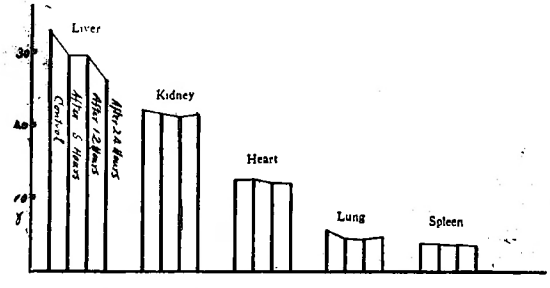
blood also decreased. And in this experiment the decrease is in the ester-form just as in the cases 6 and 12 hours after injection (Table 8). The summary of these

Table 8
Concentration of Ester-form Riboflavin in Organs and Blood Following a Single Intravenous Administration of Fat Emulsion
(Values Obtained 24 Hours after Injection)

No. of Rabbits	Liver	Kidney	Heart	Lung	Spleen	Blood
	(γ/gm)					(γ/cc)
1.	26.64(0.74)	23.41(0.64)	12.35(0.14)	5.12(0.11)	3.41(0.12)	0.130(0.02)←—0.138(0.02)
2.	28.14(0.86)	21.16(0.58)	11.85(0.15)	4.78(0.13)	3.56(0.13)	0.115(0.01)←—0.122(0.01)
3.	24.31(0.81)	21.17(0.53)	12.12(0.13)	4.86(0.15)	3.10(0.13)	0.127(0.01)←—0.145(0.02)
4.	27.14(0.90)	22.34(0.71)	11.48(0.17)	5.03(0.13)	3.42(0.14)	0.133(0.02)←—0.154(0.02)
5.	23.87(0.95)	20.35(0.75)	12.32(0.13)	4.92(0.15)	3.05(0.13)	0.121(0.01)←—0.131(0.02)
Average	26.02(0.85)	21.69(0.64)	12.02(0.14)	4.94(0.13)	3.31(0.13)	0.125(0.01)←—0.138(0.02)
Control	33.01(0.87)	22.11(0.60)	12.31(0.15)	5.31(0.12)	3.64(0.12)	0.145(0.02) *

* : Before Injection
( ) : Concentration of Free Riboflavin (F. R.)

Fig. 5
Concentration of Riboflavin in Organs of Healthy Adult Rabbits Following a Single Intravenous Administration of Fat Emulsion.



results is shown in Fig. 5. The fact that the decrease of total riboflavin and the ester-form in every organ of rabbits administered the fat emulsion is greater than that of control rabbits administered the same quantity of glucose, may explain that riboflavin participates more in the process of fat metabolism than of carbohydrate metabolism. Today is known that carbohydrate, fat and protein all enter into the common metabolism process (KREBS' T. C. A. Cycle) and are finally oxidized and all hydrogen isolated by circulation of T. C. A. Cycle is carried by Pyridine Nucleotide, Falvoprotein and Cytochrom-System and at last converted to water by combination with oxygen. In short, all nutrients are oxidized and converted to carbon dioxide and water.

Then why does the distinct decrease of riboflavin occur after intravenous administeration of fat emulsion as compared with controls? The explanation of this point seems to be as follows; According to the present generally accepted concept of fat metabolism, fatty acids first combine with CoA and are activated as mentioned above, enter into the Fatty Acid Cycle (LYNEN), then are oxidized. F. A. D. and Diphospho-Pyridine-Nucleotid (D. P. N.) are necessary for the smooth

function of the Fatty Acid Cycle.

In other words, only when the function of F. A. D. and D. P. N. as hydrogen-carriers is sufficiently displayed, the function of the Fatty Acid Cycle is smooth and fatty acids are finally converted to acetly-CoA. Therefore, during the oxidation process of fatty acids riboflavin has important physiological functions in two steps. The fact that riboflavin can participate in fat metabolism only in the form of F. A. D. is well understood from the finding that the decrease of total riboflavin in organs and blood is caused by the decrease of the ester-form in the case of intravenous administration of fat emulsion. And it is well known that the decrease of riboflavin by fat administration is most distinct in liver. This fact is regarded today as most important in the process of fat metabolism in the body.

3. Concentration of Riboflavin in Organs and Blood of Healthy Adult Rabbits Following a Single Injection of Fat Emulsion and Riboflavin.

It is evident from the results mentioned above that the consumption of riboflavin in the body is increased by administration of fat emulsion.

In order to determine how much riboflavin is necessary to prevent a deficiency of riboflavin in the body during fat administration, simultaneous injection of the fat emulsion and riboflavin-5′-phosphate in doses of 1mg, 2mg, and 5mg per kg of body weight was done, then concentration of riboflavin in organs and blood was measured hour by hour. If possible, additional injection of F. A. D is recommended also in this experiment, considering physiological functions of riboflavin in body. Riboflavin-5′-phosphate sodium solution was used, however, because at present it is difficult to obtain a pure, inexpensive form of F. A. D.. Moreover, FLEXER et al. have published information that synthetic riboflavin-5′-phosphate coincides with F. M. N..

15 per cent fat eulsion was injected intravenously in a dosage of 3.3cc per kg

Table 9
Concentration of Ester-form Riboflavin in Organs and Blood Following a Single Intravenous Administration of Fat Emulsion and Riboflavin 1mg.
(Values Obtained 6 Hours after Injection)

No. of Rabbits	Liver	Kidney	Heart	Lung	Spleen	Blood
	(γ/gm)					(γ/cc)
1.	30.56(0.95)	22.44(0.71)	12.43(0.18)	4.46(0.15)	3.61(0.12)	0.135(0.02)←—0.141(0.02)
2.	29.61(0.91)	21.76(0.67)	11.93(0.14)	4.82(0.11)	3.14(0.14)	0.128(0.01)←—0.132(0.02)
3.	30.15(0.74)	20.14(0.63)	12.44(0.16)	4.94(0.13)	3.52(0.13)	0.133(0.02)←—0.135(0.02)
4.	28.76(0.81)	21.54(0.57)	12.87(0.16)	4.37(0.13)	3.01(0.12)	0.113(0.01)←—0.117(0.01)
5.	29.53(0.82)	21.48(0.54)	12.74(0.13)	3.97(0.14)	3.57(0.13)	0.124(0.01)←—0.125(0.01)
Average	29.72(0.85)	21.47(0.62)	12.48(0.15)	4.51(0.13)	3.37(0.13)	0.127(0.01)←—0.130(0.02)
Control	31.64(0.78)	22.24(0.63)	12.53(0.14)	5.14(0.12)	3.61(0.12)	0.135(0.02) *

* : Before Injection
( ) : Concentration of Free Riboflavin (F. R.)

of body weight and riboflavin and methionin were also given subcutaneously in these experiments.

(a) Injection of Riboflavin 1mg.

The concentration of the ester-form of riboflavin in organs and blood 6, 12 and 24 hours after intravenous administration is shown in Tables 9, 10 and 11 and

Table 10

Concentration of Ester-form Riboflavin in Organs and Blood Following a Single Intravenous Administration of Fat Emulsion and Riboflavin 1 mg.
(Values Obtained 12 Hours after Injection)

No. of Rabbits	Liver	Kidney	Heart	Lung	Spleen	Blood
	(γ/gm)					(γ/cc)
1.	30.64(0.91)	21.25(0.63)	12.41(0.15)	4.57(0.15)	3.51(0.11)	0.134(0.02)←0.151(0.02)
2.	28.33(0.83)	20.22(0.70)	12.45(0.14)	4.11(0.13)	2.97(0.14)	0.116(0.01)←0.146(0.01)
3.	30.85(0.82)	21.58(0.66)	11.95(0.16)	4.96(0.11)	3.86(0.15)	0.125(0.01)←0.152(0.02)
4.	29.58(0.68)	21.03(0.55)	11.67(0.18)	4.26(0.14)	3.22(0.12)	0.135(0.02)←0.137(0.02)
5.	29.55(0.74)	21.51(0.62)	12.21(0.14)	4.33(0.13)	3.88(0.11)	0.133(0.02)←0.158(0.02)
Average	29.79(0.79)	21.12(0.63)	12.14(0.15)	4.45(0.13)	3.49(0.13)	0.129(0.02)←0.149(0.02)
Control	33.14(0.86)	21.87(0.51)	12.31(0.15)	5.32(0.12)	3.62(0.11)	0.145(0.02) *

* : Before Injection
( ) : Concentration of Free Riboflavin (F. R.)

Table 11

Concentration of Ester-form Riboflavin in Organs and Blood Following a Single Intravenous Administration of Fat Emulsion and Riboflavin 1 mg.
(Values Obtained 24 Hours after Injection)

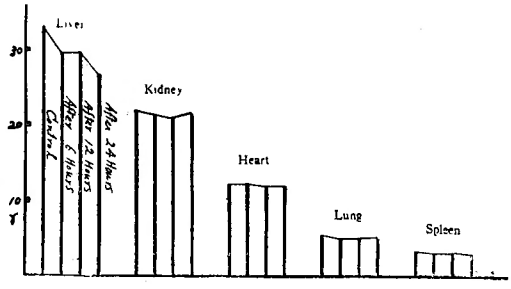
No. of Rabbits	Liver	Kidney	Heart	Lung	Spleen	Blood
	(γ/gm)					(γ/cc)
1.	24.01(0.92)	21.33(0.74)	12.33(0.15)	4.91(0.14)	3.04(0.13)	0.131(0.02)←0.137(0.02)
2.	27.84(0.90)	22.35(0.72)	11.44(0.17)	5.13(0.13)	3.42(0.14)	0.125(0.01)←0.131(0.02)
3.	24.88(0.81)	21.57(0.55)	12.21(0.13)	4.88(0.15)	2.97(0.15)	0.133(0.02)←0.140(0.02)
4.	29.14(0.86)	21.15(0.57)	11.91(0.15)	4.72(0.13)	3.57(0.13)	0.134(0.02)←0.143(0.02)
5.	26.61(0.73)	23.44(0.65)	12.55(0.14)	5.12(0.11)	3.66(0.12)	0.128(0.01)←0.132(0.02)
Average	26.49(0.84)	21.97(0.65)	12.08(0.15)	4.95(0.13)	3.33(0.13)	0.130(0.02)←0.137(0.02)
Control	34.15(0.87)	22.39(0.61)	12.51(0.14)	5.43(0.12)	3.62(0.12)	0.135(0.02) *

* : Before Injection
( ) : Concentration of Free Riboflavin (F. R.)

illustrated in Fig. 6.

The concentration of the ester-form of riboflavin in liver and blood decreased by injection of riboflavin in a dosage of 1mg per kg of body weight as the result of these experiments is shown. As in the case without riboflavin the ester-form of riboflavin in liver decreased most distinctly 24 hours after administration.

Fig. 6
Concentration of Riboflavin in Organs of Healthy Adult Rabbits Following a Single Injection of Fat Emulsion and Riboflavin 1 mg.



In short, 1mg of riboflavin per kg of body weight was not enough to replace the loss of riboflavin in organs and blood after administration of the fat emulsion.

(b) Injection of Riboflavin 2mg.

Riboflavin was measured 6, 12 and 24 hours after administration just as the case of injection of 1mg riboflavin. The results obtained are shown in Tables 12, 13, 14 and Fig. 7.

In this case, in spite of intravenous

Table 12
Concentration of Ester-form Riboflavin in Organs and Blood Following a Single Intravenous Administration of Fat Emulsion and Riboflavin 2 mg.
(Values Obtained 6 Hours after Injection)

No. of Rabbits	Liver	Kidney	Heart	Lung	Spleen	Blood
	(γ/gm)					(γ/cc)
1.	32.14(0.81)	21.37(0.53)	12.30(0.13)	5.08(0.14)	3.41(0.13)	0.157(0.02)←—0.161(0.02)
2.	31.71(0.92)	20.51(0.63)	11.96(0.14)	4.98(0.13)	3.21(0.12)	0.144(0.02)←—0.145(0.02)
3.	30.57(0.82)	21.44(0.52)	12.34(0.16)	5.12(0.11)	3.58(0.14)	0.132(0.01)←—0.135(0.02)
4.	34.31(0.85)	22.71(0.61)	12.42(0.14)	5.34(0.13)	3.86(0.14)	0.161(0.02)←—0.160(0.02)
5.	30.24(0.81)	21.38(0.71)	12.56(0.13)	5.38(0.13)	3.46(0.12)	0.152(0.02)←—0.158(0.02)
Average	31.79(0.84)	21.48(0.60)	12.32(0.14)	5.18(0.13)	3.50(0.13)	0.149(0.02)←—0.152(0.02)
Control	31.86(0.92)	22.14(0.61)	12.41(0.15)	5.11(0.12)	3.64(0.11)	0.151(0.02) *

* : Before Injection
( ) : Concentration of Free Riboflavin (F. R.)

Table 13
Concentration of Ester-form Riboflavin in Organs and Blood Following a Single Intravenous Administration of Fat Emulsion and Riboflavin 2 mg.
(Values Obtained 12 Hours after Injection)

No. of Rabbits	Liver	Kidney	Heart	Lung	Spleen	Blood
	(γ/gm)					(γ/cc)
1.	31.54(0.83)	21.82(0.64)	12.15(0.13)	5.02(0.15)	3.42(0.12)	0.134(0.02)←—0.132(0.02)
2.	33.41(0.90)	21.04(0.58)	12.68(0.15)	5.81(0.13)	3.87(0.13)	0.142(0.02)←—0.143(0.02)
3.	30.41(0.87)	20.68(0.63)	11.87(0.15)	4.92(0.13)	3.24(0.11)	0.153(0.02)←—0.151(0.02)
4.	32.59(0.82)	21.30(0.63)	12.52(0.14)	5.62(0.11)	3.62(0.13)	0.142(0.02)←—0.138(0.02)
5.	35.41(0.85)	22.14(0.71)	12.81(0.18)	5.87(0.13)	4.10(0.11)	0.131(0.01)←—0.133(0.01)
Average	32.67(0.85)	21.39(0.64)	12.41(0.15)	5.45(0.13)	3.65(0.12)	0.140(0.02)←—0.139(0.02)
Control	32.51(0.81)	21.87(0.62)	12.38(0.14)	5.38(0.14)	3.70(0.12)	0.145(0.01) *

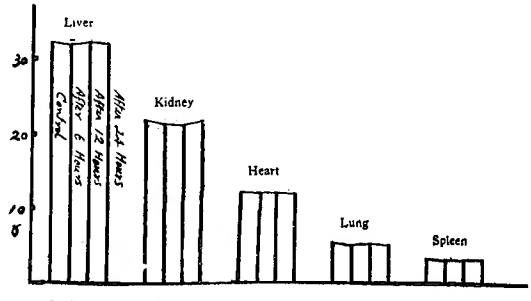
* : Before Injection
( ) : Concentration of Free Riboflavin (F. R.)

Table 14
Concentration of Ester-form Riboflavin in Organs and Blood Following a Single Intravenous Administration of Fat Emulsion and Riboflavin 2 mg.
(Values Obtained 24 Hours after Injection

No. of Rabbits	Liver	Kidney	Heart	Lung	Spleen	Blood
	(γ/gm)					(γ/cc)
1.	31.52(0.91)	21.31(0.54)	12.36(0.14)	5.12(0.12)	3.74(0.12)	0.152(0.02)←0.152(0.02)
2.	34.23(0.88)	23.58(0.66)	12.46(0.16)	5.37(0.14)	3.98(0.14)	0.160(0.02)←0.158(0.02)
3.	30.24(0.75)	20.96(0.74)	11.96(0.14)	4.92(0.13)	3.12(0.13)	0.132(0.01)←0.135(0.01)
4.	31.59(0.87)	21.52(0.63)	12.14(0.15)	5.21(0.12)	3.62(0.13)	0.135(0.02)←0.137(0.02)
5.	33.02(0.76)	22.76(0.64)	12.51(0.13)	5.29(0.13)	3.88(0.14)	0.141(0.02)←0.138(0.02)
Average	32.14(0.83)	22.03(0.64)	12.29(0.14)	5.18(0.13)	3.67(0.13)	0.144(0.02)←0.144(0.02)
Control	32.48(0.86)	23.01(0.70)	12.34(0.14)	5.22(0.13)	3.71(0.12)	0.151(0.02) *

* : Before Injection
( ) : Concentration of Free Riboflavin (F. R.)

Fig. 7
Concentration of Riboflavin in Organs of Healthy Adult Rabbits Following a Single Injection of Fat Emulsion and Riboflavin 2 mg.



administration of the fat emulsion, the concentration of the ester-form of riboflavin in organs and blood remained normal in every case and a difference from controls could not be found. In short, simultaneous use of methionin and riboflavin in a dosage of 2mg per kg of body weight can probably replace the loss of riboflavin caused by intravenous administration of the fat emulsion.

(c) Injection of Riboflavin 5mg.

Riboflavin was measured 6, 12 and 24 hours after administration of the fat emulsion, methionin and riboflavin 5mg per kg of body weight.

The results obtained are shown in Tables 15, 16, 17 and Fig. 8. The decrease of the ester-form of riboflavin in organs and blood was not consistently found. A slight increase in liver and kidney riboflavin was noticed but an increase of this degree is of little significance when compared with the results of injection of 2mg riboflavin. Excessive riboflavin is probably entirely excreted in urine. Considering these results, the optimum dosage of riboflavin necessary to use with methionin seems to be about 2mg per kg of body weight during intravenous administration of 15 per cent fat emulsion in dosage of 3.3cc per kg of body weight.

4. Concentration of Riboflavin in Organs and Blood of Healty Adult Rabbits after Repeated Intravenous Administration of Fat Emulsion.

Table 15

Concentration of Ester-form Riboflavin in Organs and Blood Following a Single Intravenous Administration of Fat Emulsion and Riboflavin 5 mg.

(Values Obtained 6 Hours after Injection)

No. of Rabbits	Liver	Kidney	Heart	Lung	Spleen	Blood
	(γ/gm)					(γ/cc)
1.	32.51(0.91)	21.41(0.57)	12.41(0.13)	5.21(0.12)	3.41(0.11)	0.134(0.02)←—0.140(0.02)
2.	31.74(0.83)	20.14(0.51)	11.92(0.14)	5.11(0.14)	3.23(0.12)	0.131(0.01)←—0.135(0.02)
3.	34.58(0.87)	22.86(0.61)	12.58(0.13)	5.72(0.12)	3.92(0.12)	0.142(0.02)←—0.147(0.02)
Average	32.94(0.87)	21.47(0.56)	12.30(0.13)	5.32(0.13)	3.52(0.13)	0.136(0.02)←—0.141(0.02)
Control	32.76(0.91)	21.86(0.61)	12.41(0.12)	5.41(0.12)	3.41(0.11)	0.137(0.02) *

* : Before Injection

( ) : Concentration of Free Riboflavin (F. R.)

Table 16

Concentration of Ester-form Riboflavin in Organs and Blood Following a Single Intravenous Administration of Fat Emulsion and Riboflavin 5 mg.

(Values Obtained 12 Hours after Injection)

No. of Rabbits	Liver	Kidney	Heart	Lung	Spleen	Blood
	(γ/gm)					(γ/cc)
1.	31.72(0.84)	20.83(0.64)	11.92(0.14)	5.12(0.14)	3.52(0.11)	0.135(0.01)←—0.134(0.01)
2.	34.31(0.87)	21.34(0.59)	12.54(0.13)	5.74(0.12)	3.64(0.12)	0.151(0.02)←—0.150(0.02)
3.	35.14(0.92)	22.30(0.70)	12.64(0.14)	5.81(0.12)	3.81)0.11)	0.152(0.02)←—0.142(0.02)
Average	33.72(0.88)	21.48(0.64)	12.36(0.14)	5.56(0.13)	3.66(0.11)	0.146(0.02)←—0.142(0.02)
Control	32.86(0.82)	21.56(0.70)	12.34(0.13)	5.42(0.12)	3.58(0.11)	0.142(0.02) *

* : Before Injection

( ) : Concentration of Free Riboflavin (F. R.)

Table 17

Concentration of Ester-form Riboflavin in Organs and Blood Following a Single Intravenous Administration of Fat Emulsion and Riboflavin 5 mg.

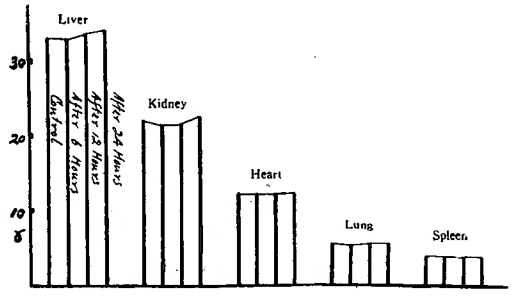
(Values Obtained 24 Hours after Injection)

No. of Rabbits	Liver	Kidney	Heart	Lung	Spleen	Blood
	(γ/gm)					(γ/cc)
1.	32.87(0.86)	21.32(0.57)	12.41(0.13)	5.10(0.12)	3.46(0.11)	0.143(0.02)←—0.151(0.02)
2.	35.26(0.81)	23.62(0.61)	12.78(0.14)	5.82(0.14)	4.12(0.14)	0.152(0.02)←—0.154(0.02)
3.	34.71(0.90)	22.85(0.64)	12.15(0.14)	5.24(0.14)	3.84(0.12)	0.144(0.02)←—0.161(0.02)
Average	34.28(0.86)	22.59(0.61)	12.45(0.14)	5.39(0.13)	3.81(0.12)	0.146(0.02)←—0.155(0.02)
Control	33.42(0.91)	22.64(0.56)	12.38(0.14)	5.37(0.13)	3.68(0.12)	0.148(0.02) *

* : Before Injection

( ) : Concentration of Free Riboflavin (F. R.)

Fig. 8
Concentration of Riboflavin in Organs of Healthy Adult Rabbits Following a Single Injection of Fat Emulsion and Riboflavin 5mg.



As the above results were obtained by only a single administration of the fat emulsion, it is obviously impossible to determine conclusively the relationship between the quantity of administered fat emulsion and that of consumption of riboflavin used by fat administration by these results alone.

Therefore, a measure of the concentration of riboflavin in organs and blood of rabbits aministered fat emulsion for 3 consecutive weeks was done. Also the quantity of riboflavin and simultaneous injection of methionin, pantothenic acid, nicotinic acid and vitamin C in various combinations were done. Because, as mentioned previously, enzyme systems such as CoA, Flavoprotein and Pyridin Nucleotide etc. participating in the fat metabolism cosist of vitamins as pantothenic acid, riboflavin and nicotinic acid, and vitamin C has a function of activating aconitase and dehydrogenase system of succinic acid and is also a important vitamin for the fat metabolism. Rabbits in 5 groups of 3 animals each were administered the fat emulsion as described below for 3 consecutive weeks, killed by exsanguination and then the concentration of riboflavin in organs and blood was measured.

1st group : fat (0.5g)* +riboflavin-5′-phosphate(2mg)* +*l*-methionin (5mg)*
2nd group : fat (0.5g)* +*l*-methionin(5mg).*

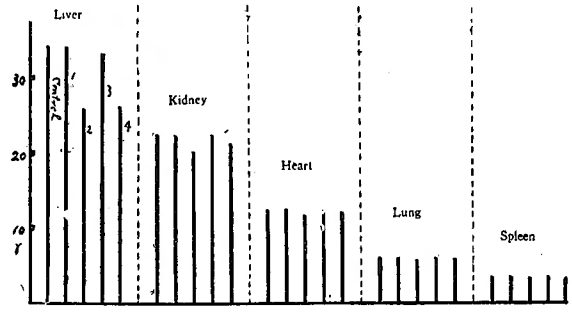
Table 18
Concentration of Ester-form Riboflavin in Organs and Blood of Healthy Adult Rabbits after Repeated Intravenous Administration of Fat Emulsion.

Group	Liver	Kidney	Heart	Lung	Spleen	Blood
	(γ/gm)					(γ/cc)
1.	34.01	22.46	12.72	5.61	3.82	0.133←—0.137
2.	25.86	20.46	11.56	5.21	3.46	0.127←—0.141
3.	33.26	22.38	12.46	5.92	3.46	0.142←—0.145
4.	26.14	21.22	11.96	5.42	3.16	0.128←—0.138
Control	34.26	22.81	12.47	5.88	3.68	0.146←—0.151
						*

1 st. Group : Fat + Riboflavin − 5′ − phosphate + *l* − Methionine.
2 nd. Group : Fat + *l* − Methionine.
3 rd. Group : Fat + Riboflavin − 5′ − phosphate + *l* − Methionine + Niacin amide + Calcium pantothenate + Thiamin hydrochloride + *l* − Ascorbic acid.
4 th. Group : Fat + *l* − Methionine + Niacin amide + Calcium pantothenate + Thiamin hydrochloride + *l* − Ascorbic acid.
* : Before Injection

* ( ) is a injected dosage per kg of body weight per day.

Fig. 9
Concentration of Riboflavin in Organs of Healthy Adult Rabbits after Repeated Intravenous Administration of Fat Emulsin.



3rd group : fat (0.5g)* +*l*-methionin (5mg)* +riboflavin-5′-phosphate (2mg)* +niacin amide (4mg)* +calcium pantothenate (5mg)* +thiamin hydrochloride (1mg)* +*l*-ascorbic acid (10mg).*

4th group : fat (0 5g)* +*l*-methionin (5mg)* +niacin amide (4mg)* + calcium pantothenate (5mg)* + thiamin hydrochloride (1mg)* +*l*-ascorbic acid(10mg).*

5th group (control) : glucose (0.23g).*

The average values obtained in each group are shown in Table 18 and Fig. 9.

Surveying the results, the concentration of ester-form riboflavin in liver, kidney and heart decreased in the 2nd, 4th group without use of riboflavin, the decrease in liver being most marked. Riboflavin in blood showed a tendency to decrease a little. But in lung and spleen, a significant decrease of riboflavin was not found. On the other hand, a slight decrease of riboflavin in organs and blood was found in the 1st and 3rd group which used riboflavin during intravenous administration of the fat emulsion whether or not there was simultaneous use of vitamins other than riboflavin. It seems clear that the necessary dosage of riboflavin during repeated administration is 2mg per kg of body weight just as with single administration of fat to healthy adult rabbits.

The decrease of riboflavin in kidney and heart was relatively distinct in the 2nd and 4th group which did not receive riboflavin, differing from the results of a single injection. This fact may probably mean that kidney and muscle play a great part in the fat metabolism when intravenous administration of the fat emulsion is continued for a long time even if triglycerides of long chain fatty acids are used in the fat emulsion. Phospholipids produced by administration of the emulsion which was contained triglyceride of long chain fatty acids, can enter directly into kidney and muscles and be oxidized. Also a part of fatty acids entering into parenchymal cells of liver in the form of phospholipids can be oxidized only to the stage of keton bodies in liver. Therefore they are again transported to kidney and muscles by blood and then can be finally oxidized there. It seems that riboflavin has an important function as a hydrogen-carrier in the form of F. A. D. during intravenous administration of the fat emulsion both when it enters into the Fatty Acid Cycle and functions in this cycle and when it enters into T. C. A. Cycle as acetyl CoA conjugated with oxalo-acetic acid and functions in this cycle. This fact is very interesting comparing with the recent experiments of NATH and CHAKRABARTI that rabbits receiving continuous injection of sodium acetoacetate and β-hydroxy-butyrate for 3 months show a state of riboflavin and nicotinic acid deficiency.

5. Concentration of Riboflavin in Organs and Blood of Rabbits Fed Subcalori-

cally after Repeated Intravenous Administration of Fat Emulsion.

In practice, the objective of intravenous administration of fat emulsion is obviously the malnourished patient. Therefore 15 per cent fat emulsion in a dosage of 3.3cc per kg of body weight was injected intravenously into rabbits fed only one third of the standard diet for 3 consecutive weeks. The rabbits were killed by exsanguination and the concentration of ester-form riboflavin was measured in organs and blood.

The diet consisted of bran, 40g, raw radish leaves, 40g and water, 40cc daily. There were 5 groups of rabbits each consisting of 3 rabbits like the above experiment. Averaged values of each group are shown in Table 19 and Fig. 10. A decrease of riboflavin in liver, kidney and heart was found in the 2nd and 4th groups which did not receive riboflavin. The decrease in liver was outstanding. And the degree of decrease tended to be a little greater than that of rabbits fed by standard diet above mentioned.

This tendency was seen to only a slight degree in the lst and 3rd groups which received riboflavin. It is characteristic in this experiment that riboflavin in organs, especially

Table 19

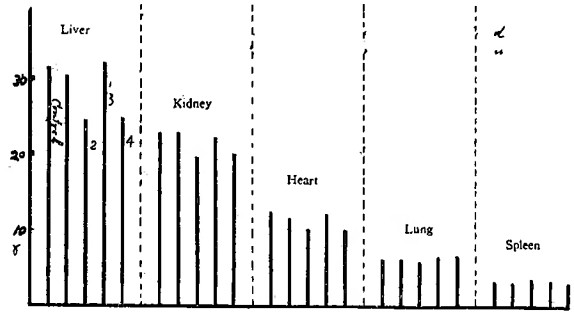
Concentration of Ester-form Riboflavin in Organs and Blood of Rabbits Fed Subcalorically after Repeated Intravenous Administration of Fat Emulsion

Group	Liver	Kidney	Heart	Lung	Spleen	Blood
	(γ/gm)					(γ/cc)
1.	30.36	22.93	11.43	5.00	3.08	0.141←—0.138
2.	25.49	19.61	10.62	4.94	3.63	0.132←—0.135
3.	32.54	22.34	12.15	5.52	3.41	0.142←—0.141
4.	25.81	20.16	10.81	5.41	3.15	0.134←—0.140
Control	31.48	22.62	12.41	5.02	3.24	0.130←—0.138
						*

1st. Group : Fat + Riboflavin-5′-phosphate + *l*-Methionine.
2nd. Group : Fat + *l*-Methionine.
3rd. Group : Fat + Riboflavin-5′-phosphate + *l*-Methionine + Niacin amide + Calcium pantothenate + Thiamin hydrochloride + *l*-Ascorbic acid.
4th. Group : Fat + *l*-Methionine + Niacin amide + Calcium pantothenate + Thiamin hydrochloride + *l*-Ascorbic acid.
* : Before Injection

Fig. 10

Concentration of Riboflavin in Organs of Rabbits Fed Subcalorically after Repeated Intravenous Administration of Fat Emulsion.



in liver of control rabbits (5th group) decreases more distinctly than the control rabbits (5th group) fed by standard diet as shown in Table 18. This may be caused by the fact that administration of glucose in this amount not supply the necessary calories lacking in the dificient diet and therefore glycogen storage decreases gradually and then fat storage is mobilized into the blood and oxidized. The greater decrease of riboflavin in organs of this low caloric experiment as compared with the standard diet experiments may be caused by the quantity of riboflavin in the diet and also by mobilization and utilization of fat storage and further by increase of riboflavin excretion in urine caused by destruction of protein. It seems therefore that the use of riboflavin at least in a slightly larger dosage than 2mg per kg of body weight may improve the repeated intravenous administration of fat emulsion.

6. Quantity of Riboflavin Excreted in Urine of Fasted Rabbits Following Intravenous Administration of Fat Emulsion.

Rabbits were first fed a standard diet for a fixed period and simultaneously injected daily with vitamins necessary for fat metabolism. Rabbits were divided into F-group, G-group and FG-group, each consiting of 3 rabbits. All groups fasted at the same time, and daily intravenous injections of fat were made in a dosage of 0.5g per kg of body weight (15 per cent fat emuslion 3.3cc) to F-group, glucose 0.5g to G-group, fat 0.5g and glucose 0.5g to FG-group with vitamins as above mentioned. The total quantity of urine per day consisted of catheterized urine plus urine which accumulated in a bottle below the feeding cage. Brown bottles were used to avoid light and the urine was kept acid by acetic acid and the concentration of riboflavin in urine measured every other day. In this experiment fat emulsion without glucose was used. As shown in Table 20 and Fig. 11, in every day of fast, the quantity of riboflavin excreted in urine of the control animals was the greatest and became less in the order of G-group, F-

Table 20

Quantity of Ester-form Riboflavin Excreted in Urine of Fasted Rabbits Following Intravenous Administration of Fat Emulsion

Group	F	G	FG	Control
Before Injection	869.19 γ	857.05 γ	873.53 γ	862.95 γ
1-2 Days after Inj.	1008.28 γ	1108.64 γ	928.51 γ	1368.76 γ
3-4 Days after Inj.	988.39 γ	1170.86 γ	965.15 γ	1309.33 γ
5-6 Days after Inj.	885.15 γ	965.19 γ	862.26 γ	1157.78 γ

F Group : Fat + Riboflavin-5′-phosphate(2mg.) + Niacin amide(4mg.) + Calcium pantothenate (5mg.) + *l*-Ascorbic acid(10mg.) + Thiamin hydrochloride(1mg.)

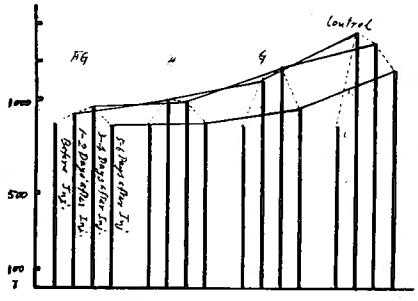
G Group : Glucose + Riboflavin-5′-phosphate(2mg.) + Niacin amide(4mg.) + Calcium pantothenate(5mg.) + *l*-Ascorbic acid(10mg.) + Thiamin hydrochloride(1mg.)

FG Group : Fat + Glucose + Riboflavin-5′-phosphate(2mg.) + Niacin amide(4mg.) + Calcium pantothenate(5mg.) + *l*-Ascorbic acid(10mg.) + Thiamin hydrochloride(1mg)

Control Riboflavin-5′-phosphate(2mg.) + Niacin amide(4mg.) + Calcium pantothenate(5mg.) + *l*-Ascorbic acid(10mg.) + Thiamin hydrochloride(1mg.)

( ) : Injected Dosage of Vitamins per kg per Day.

Fig. 11
Quantity of Riboflavin Excreted in Urine of Fasted Rabbits Following Intravenous Administration of Fat Emulsion



group and FG-group. Glycogen storage in the body may be exhausted by fasting resulting in the deficiency of "*Sparker*" which is necessary for oxidation of fat, and in deficiency of A. T. P., further of oxalo-acetic acid which is necessary to carry acetyl CoA in KREBS' T. C. A. Cycle. This may be prevented by simultaneously using glucose during intravenous administration of fat emulsion. Fatty acids can not be reactivated for the sake of deficiency of A. T. P. even if CoA is sufficiently supplied, and they can not enter smoothly into the Fatty Acid Cycle. Acetyl CoA also can not enter smoothly into KREBS' T. C. A. Cycle and then are excreted in urine as ketone bodies. Therefore the consumption of riboflavin in the body may be greater in FG-group than in F-group, and consequently the quantity of riboflavin in urine may be less in FG-group than in F-group. The fact that the quantity of riboflavin in urine in F-group is less than in G-group might be explained as follows : the greater part of acetyl CoA is excreted in urine as keton bodies. But a certain portion of fat produces acetyl CoA via Fatty Acid Cycle and a part of this acetyl CoA can enter into KREBS' T. C. A. Cycle by conjugation with oxalo-acetic acid reduced from amino acids and can then be oxidized. Therefore more riboflavin is used in the body by the quantity which is necessary in the Fatty Acid Cycle. It may be expected that the demand upon riboflavin of G-group is naturally greater than the controls because glucose can finally enter into the T. C. A. Cycle and be oxidized like fat. The fact that the quantity of riboflavin in urine increases temporarily after fasting and then decreases, this tendency being significant in diminishing order in FG-group, F-group, G-group and control group, may be explained as the result of destruction of body protein.

IV. SUMMARY

ASADA, SENŌ, NAKATA, TSUKADA, HASHINO and OSA of our laboratory have shown already that the administration of fat emulsion alone is not sufficient for the organism to utilize fat efficiently, but simultaneous use of methionin, riboflavin, pantothenic acid, nicotinic acid and vitamin C is necessary during intravenous administration of the fat emulsion prepared in our laboratry. Using a different approach, only riboflavin was selected from these vitamins, the concentration of riboflavin measured in organs and blood following the intravenous administration of fat emulsion assuming a demand of riboflavin to be necessary for utilization of this fat emulsion by the organism. According to the results of these experiments, riboflavin participated in fat metabolism as the ester-form of riboflavin in vivo.

Action in vitro has already been shown by GREEN, OCHOA, LYNEN and LIPMANN et al. And the fact that the consumption of riboflavin during intravenous administration of this fat emulsion is highest in liver, kidney and muscles may mean that

these organs play the greatest part in the process of management of this fat emulsion in the body. We can well understand how indispensable is a use of riboflavin to intravenous administration of fat emulsion, from the fact that ester-form riboflavin of these organs used by intravenous administration of fat emulsion is sufficiently supplied with simultaneous injection of riboflavin-5′-phosphate. The demand of riboflavin is far greater in fat than in carbohydrate, in spite of the fact that both carbohydrate and fat enter finally into KREBS' T. C. A. Cycle and are oxidized to carbon dioxide and water. Then it can be assumed that riboflavin plays the greater part not only in it's cycles as hydrogen-carrier but in the former process of metabolism. According to the general concept of fat metabolism as explained by LYNEN et al., the function of riboflavin as F. A. D. in the Fatty Acid Cycle can not be ignored. But comparing the recent results of NATH and CHACRABARTI that rabbits receiving continuous injection of sodium acetoacetate and β-hydroxy-butyrate for 3 months show a distinct decrease of riboflavin and nicotinic acid in blood and urine, it can be easily assumed that riboflavin performs important physiological functions in the process of fat metabolism in two stages.

V. CONCLUSION

I measured the concentration of total riboflavin and the ester-form of riboflavin in organs, blood and urine following intravenous administration of fat emulsion produced in our laboratory and obtained the following conclusions.

(1) The concentration of riboflavin in organs and blood decreases after intravenous administration of this fat emulsion, and this is caused by the decrease of ester-form riboflavin. The decrease in liver, kindney and heart muscle is especially marked.

(2) Simultaneous use of riboflavin-5′-phosphate in a dosage of 2mg per kg of body weight seems most adequate for intravenous administration of 15 per cent fat emulsion (sesame-oil) in a dosage of 3.3cc per kg of body weight. A slightly higher dosage seems to indicate for subcaloric nutrition.

(3) Riboflavin, which must be used during intravenous administration of this fat emulsion is theoretically F. A. D., but even F. M. N. can produce the same results.

(4) From the fact that among lung, liver and spleen all of which are able to phagocytize administered fat globules and convert neutral fat to phospholipids, only the former two show the decrease of ester-form riboflavin and the latter does not show entirely the decrease, it can not be considered that the action point of riboflavin consists in converting neutral fat to phospholipids. The action point of riboflavin does seem to consist in the further process of oxidation of fat.

(5) It can be assumed that riboflavin participates in the oxidation of administered fat as the ester-form.

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経静脈性脂肪輸入時に於ける生体内リボフラビン含有量の消長に関する実験的研究

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我々の教室で創製した経静脈性輸入可能な脂肪乳剤を用いて，生体内脂質代謝過程に於けるリボフラビンの役割について研究し，次の如き結論に到達した．

(1)生体内に於て脂質代謝が著明に亢進する時は，生体内VB_2の消費量は高まり，それに伴い血液，臓器中の総VB_2量は減少する．

(2)この総VB_2量の減少はエステル型VB_2量の減少にもとづくもので，而もその減少程度は肝，腎，心に於て特に著明であつた．

(3)15%胡麻油乳剤を健常家兎の静脈内へ体重毎瓩当り3.3ccの割合で注入する時は，Rifoflavin-5′-phosphate毎瓩当り2mgの併用が望ましい．併し減食家兎に注入する際には，これよりも稍々増量して併用することが必要と思われる．

(4)又本脂肪乳剤の注入に際して併用すべきリボフラビンとしては，理論的にはF. A. D. を以てするのを理想的とするが，F. M. N. を以てしても充分その目的を達する．

(5)従つて注入脂質の酸化に際し，リボフラビンはエステル型VB_2としてその酸化に関与しているものと推察される．

(6)脂肪乳剤の静脈内注入に際して，特に肝，腎，心のエステル型VB_2量の消費が高まる事実は，リボフラビンの作用点が中性脂肪の Phospholipid 化にあるのではなく，それ以降の脂肪酸酸化機転にあるものといい得る．